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Madreporite Function and Fluid Volume Relationships in Sea Urchins

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Abstract. An effort was made to demonstrate an influx of seawater through the madreporites of sea urchins and to evaluate how such an influx, along with osmotic differences and other factors, could contribute to fluid homeostasis. Fluorescent microbeads placed in the medium of *Strongylocentrotus droebachiensis* were taken up into the pore canals and stone canal and distributed (in small numbers) to the distal tube feet, confirming a slow bulk inflow of seawater through the madreporite, where it is partially purified. Probably none of this fluid stream is diverted to the perivisceral coelom (as it is in asteroids), since experiments with *Strongylocentrotus pallidus* showed no significant movement of a soluble fluorescent tracer into that compartment. The osmotic concentrations of the perivisceral coelomic fluids in these two species, and in *Strongylocentrotus franciscanus*, were higher than that of ambient seawater by 2.66 $\pm$ 0.39 mosmol kg^{-1} (mean $\pm$ SE). That small hyperosmoticity, along with the net hydrostatic pressure differences induced by the flexing peristome, probably stabilizes body fluid volume. Likewise, fluid in the tube feet of *S. franciscanus* is elevated by 7.94 $\pm$ 1.04 mosmol kg^{-1} above seawater, which should contribute to their inflation. Blockage of the madreporite does not lead to an obviously reduced activity of the tube feet, but over the long term, an influx through the madreporite is necessary. Specimens of *S. droebachiensis* with an obstructed madreporite, fed *ad libitum*, had significantly ($P < 0.006$) reduced gut contents *vs* controls after 28 days, indicating impaired movement or feeding; and the body weights (*i.e.*, volumes) of unfed specimens were significantly ($P < 0.013$) more reduced after 21 days. Compared to starfish, the rigid test of sea urchins reduces the need for an influx of seawater through the madreporite, but some small admission is still essential.

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Introduction

Sea urchins, like many other echinoderms, possess a conspicuous sieve-like opening to the exterior, the madreporite. This structure might be expected to have a function in fluid volume regulation, but no such role has ever been demonstrated. The madreporite is attached to a ciliated "stone canal" that connects through a series of passages to the animal's fluid-filled appendages, the tube feet. Together, these structures constitute the water vascular system, an important distinguishing feature of the phylum.

Observers often assume that seawater is drawn in through the madreporite by the stone canal cilia to hydraulically extend the tube feet. If, however, the connection to the madreporite is removed, the tube feet still continue to function for a long time; I have observed tube feet on broken pieces of sea urchin tests to remain distended and active for days. Further, the fluid within the water vascular system (ambulacral fluid, or AF) of echinoderms is not exactly identical in composition to seawater (Robertson, 1949; Binyon, 1964, 1966, 1976; Prusch, 1977; Ferguson, 1987).

Sea urchins have not been studied adequately, but precise osmotic measurements have been made on the fluid compartments of starfish (asteroids). Ambulacral fluid from starfish is sufficiently hyperosmotic to ambient seawater (about 6 mosmol kg^{-1}) that some water would be drawn into this compartment from the medium, as well as from the perivisceral coelomic fluid (PCF) which is variably 1.5 mosmol kg^{-1} more concentrated than sea-

water (Ferguson, 1990a). But in addition to this osmotic uptake, studies with fluorescent microbeads demonstrate that seawater does flow into the madreporite pores of asteroids (Ferguson, 1990b) and, to a lesser extent, of ophiuroids (Ferguson, 1995), and that it is distributed to peripheral parts of the water vascular system. When soluble dextran tracer was used to measure the flux of seawater into a starfish (*Echinaster graminicola*) through the madreporite, the rate was found to be about 5.5% of the body weight per day (Ferguson, 1989). However, this study also revealed that much of this inflow was diverted from the water vascular system to the perivisceral coelom, thus maintaining the fluid volume of the flexible body. (The Tiedemann's bodies that bulge from the ring canal of asteroids into the perivisceral coelomic space were probably the main route of the diversion (Ferguson, 1990b).) Further investigation showed that some starfish, such as the intertidal *Pisaster ochraceus* with its nearly impermeable integument, appear to rely heavily on transmadreporitic entry of seawater to maintain fluid volume (Ferguson, 1992), whereas others, such as the soft-bodied *Pycnopodia helianthoides,* may be proportionally more dependent on osmotic entry of seawater through a thin, permeable body wall (Ferguson 1990a, 1994). A balance between physical uptake of seawater through the madreporite and osmotic uptake through the integument appears to be typical for asteroids.

Sea urchins (regular echinoids), like asteroids, also have prominent madreporites, but their bodies are rigid. This latter feature would seem, at first, to obviate the need for most adjustments of body fluid volume or for any special mechanism (such as the madreporite) to take up seawater. On the other hand, the flexible peristome may allow for more variation in body fluid volume than has been appreciated, creating a potential need for fluid uptake. Or, the body fluid osmotic relationships of sea urchins may not be the same as those described in starfish (Ferguson, 1990a, 1992), and not adequate to maintain fluid homeostasis. So there is much uncertainty, and unfortunately literature bearing on these issues is desultory and often conflicting.

The most concerted previous attempt to evaluate the role of the madreporite in sea urchins was that of Fechter (1965; see review of Nichols, 1966), who glued small capillary tubes to the madreporites of five urchins (*Echinus esculentus*) so that, by observing the movement of tiny air bubbles in the tubes, he could measure any influx or efflux of fluid through them. Though his apparatus was reported as sensitive to a change of 0.8 μl, he could detect no net movement of fluid over 24 h. Nevertheless, strong mechanical or chemical stimulation would cause all of the tube feet to contract simultaneously for a prolonged period, and a "slow" fluid outflow totaling 4 to 5 μl was seen. During such episodes, the PCF hydrostatic pressure rose 15–18 mm water (about 150–180 Pa) and remained elevated for about 10 h. Through these and other experiments, as well as on theoretical grounds, Fechter concluded that the primary role of the madreporite is to maintain an even pressure balance between the AF and ambient seawater, so that when the tube feet contract en masse, the displacement of their associated ampullae into the perivisceral coelom would not impart too much pressure on the peristome. Although a limited volume of fluid can be released through the madreporite, as Fechter (1965) suggested, prolonged contraction of all the tube feet is not natural; normally (except for momentary responses) when some tube feet contract, others are extending. Fechter's (1965) failure to see any seawater entry into the madreporite is important, but should be verified.

A more detailed study of the internal fluid pressures of *Strongylocentrotus purpuratus* and *Lytechinus variegatus* was carried out by Ellers and Telford (1992). They inserted a hypodermic needle attached to a pressure transducer through the peristome and recorded the pressure fluctuations associated with simultaneous contractions of the tube feet, but these were brief and only about 4% of the magnitude reported by Fechter (1965). Periodic peristomial movements (induced by the lantern) produced a fluctuating pressure change within the perivisceral coelom of about the same magnitude; the internal pressure tended to be negative (to −8.2 Pa) 70% of the time. Although not mentioned by them, this important finding points to another probable mechanism of PCF homeostasis—pressure filtration of water into the perivisceral coelom from higher pressure areas, particularly from the gut and the tube feet ampullae. Their study led Ellers and Telford (1992) to question Fechter's argument that the madreporite is primarily involved in acute pressure equalization.

Fechter's contention is, furthermore, not consistent with the anatomy of the madreporite and its associated stone canal. The form of the madreporite does not suggest a simple "relief valve." In sea urchins the madreporite typically consists of 300–400 pore canals partially filled with cilia that tend to forcefully exclude particles (Tamori *et al.,* 1996). The inside diameter of each pore is about 21 μm, which is variable because the pore can contract to less than half its resting size in response to acetylcholine (Takahashi and Tamori, 1988; Takahashi *et al.,* 1991; Tamori *et al.,* 1996). Thus, the total cross-sectional space of the madreporite openings is only about 0.12 mm^2. The normal fluid exchanges through such a restricted opening are likely to be too small to affect the fluid volume of the animal except, perhaps, over the long term. Nevertheless, fairly large fluid volume changes (milliliters) can take place in sea urchins within hours in response to osmotic challenges (Lange, 1964; Stickle and

Ahokas, 1974). Logic suggests that this last adjustment must involve an initial displacement of the peristome, followed by rapid water and ion movements across the gut and other permeable surfaces. Moreover, in preliminary work leading to the present investigation, sea urchins (*Strongylocentrotus pallidus* and *S. purpuratus*) with obstructed madreporites were repeatedly able to return to a near-normal body weight (and volume) within hours after removal of 1–3 ml of their PCF, clearly showing that the madreporite is not needed for acute large-scale volume changes.

Thus, the functions of the prominent madreporite system of sea urchins remain unknown, and the normal osmotic differences that might exist between their various body fluids and the media have not been accurately measured. In this study methods previously applied to asteroids are used to examine two questions. First, does seawater enter the sea urchin madreporite, and if it does, is the quantity sufficient either to affect the function of the tube feet or to stabilize body fluid volume? Second, are osmotic differences between the internal fluids of sea urchins and the outside medium consistent, and could they contribute to inflation of the tube feet or to augmentation of general body fluid?

Materials and Methods

Treatment of specimens

Work was conducted on four congeneric species of sea urchins collected from waters around Friday Harbor, Washington: *Strongylocentrotus purpuratus, S. pallidus, S. droebachiensis,* and *S. franciscanus.* They were kept in shallow tanks with flowing seawater and fed *ad libitum* with kelp picked up along the shore. In some cases, the madreporites of "test" animals were obstructed by first scraping the structure with a needle and blotting up the soft tissue, and then sealing the area with freshly mixed, finely ground hydraulic cement. After the cement had hardened for about 10 min, the animals were returned to seawater. These plugs were tolerated very well, and they showed no sign of failure over the course of any of the experiments.

Fluorescent microbead experiments

Influxes of seawater through the madreporite and into the water vascular system were demonstrated as follows. Two small specimens of *S. droebachiensis* (3.1 and 3.4 g) were selected and placed for 5 days in a dish of aerated seawater (250 ml) to which was added 1 ml of a suspension of 0.2 μm YG "Fluoresbrite" carboxylate beads (Polysciences, Inc.). Then, after they were rinsed in seawater, the animals were cut into several parts to facilitate further handling. These were fixed overnight in 10% formalin and decalcified under refrigeration for 10 days in several changes of 5% EDTA and 10% formalin. After rinsing in tap water, pieces were selected and trimmed to suitable size for sectioning (3–5 mm diameter). Attention was focused on pieces containing the madreporite complex, the aboral half of the stone canal, and representative parts of the aboral and oral body walls with tube feet and ampullae attached. These were frozen onto stubs with Tissue-Tek OTC compound, sectioned in a cryostat to 20–50 μm, picked up on glass slides, mounted with cover slips over glycerine jelly, and examined under epifluorescence using a Nikon system. Only animals with intact madreporites were used for these observations; obstructing the madreporite would have destroyed the major area to be studied and would have precluded sectioning. Further, previous studies on asteroids and ophiuroids (Ferguson, 1990b, 1995) suggested that particles could enter the water vascular passages only with bulk flow of seawater through the madreporite pores.

Soluble fluorescent tracer experiments

An effort was made to quantify the influx of seawater through the madreporite with the soluble, high molecular weight tracer, fluorescein isothiocyanate dextran (FID), as was previously done with asteroids (Ferguson, 1989, 1994). To accomplish this, 100 mg FID (73,100) (Sigma Chemical) was dissolved in dishes of gently bubbled seawater (800 ml) containing pairs (one "test" and one unaltered "control") of smallish *S. pallidus* (81–155 g). At 24-h intervals, samples of 0.5 ml PCF were taken from the sea urchins through a 26-gauge hypodermic needle inserted through the peristome. Unlike asteroids, an adequate AF sample could not be obtained from the small lamellar ampullae of these animals, and larger sea urchins (including *S. franciscanus*) require so much medium that work with them is impractical. For analysis of the PCF, the collected fluid was centrifuged, and 0.1-ml aliquots of the supernatant were vortexed in tubes with 3.5 ml phosphate buffer. The fluorescence of the samples in these tubes was read in a Turner fluorimeter equipped with 2A and 47B primary filters and a 2A-12 secondary filter. Measurements were compared with samples of medium that had been similarly processed, as well as samples of medium that were diluted 1:10. This method can detect FID in the PCF to concentrations about 0.25% that of the medium.

Long-term effects of madreporite obstruction

Two sets of experiments tested the effect of long-term obstruction of the madreporite on behavior, tube feet activity, and maintenance of body fluid volume. It was assumed that small, gradual adjustments in the volume of individual sea urchins would be observable as systematic

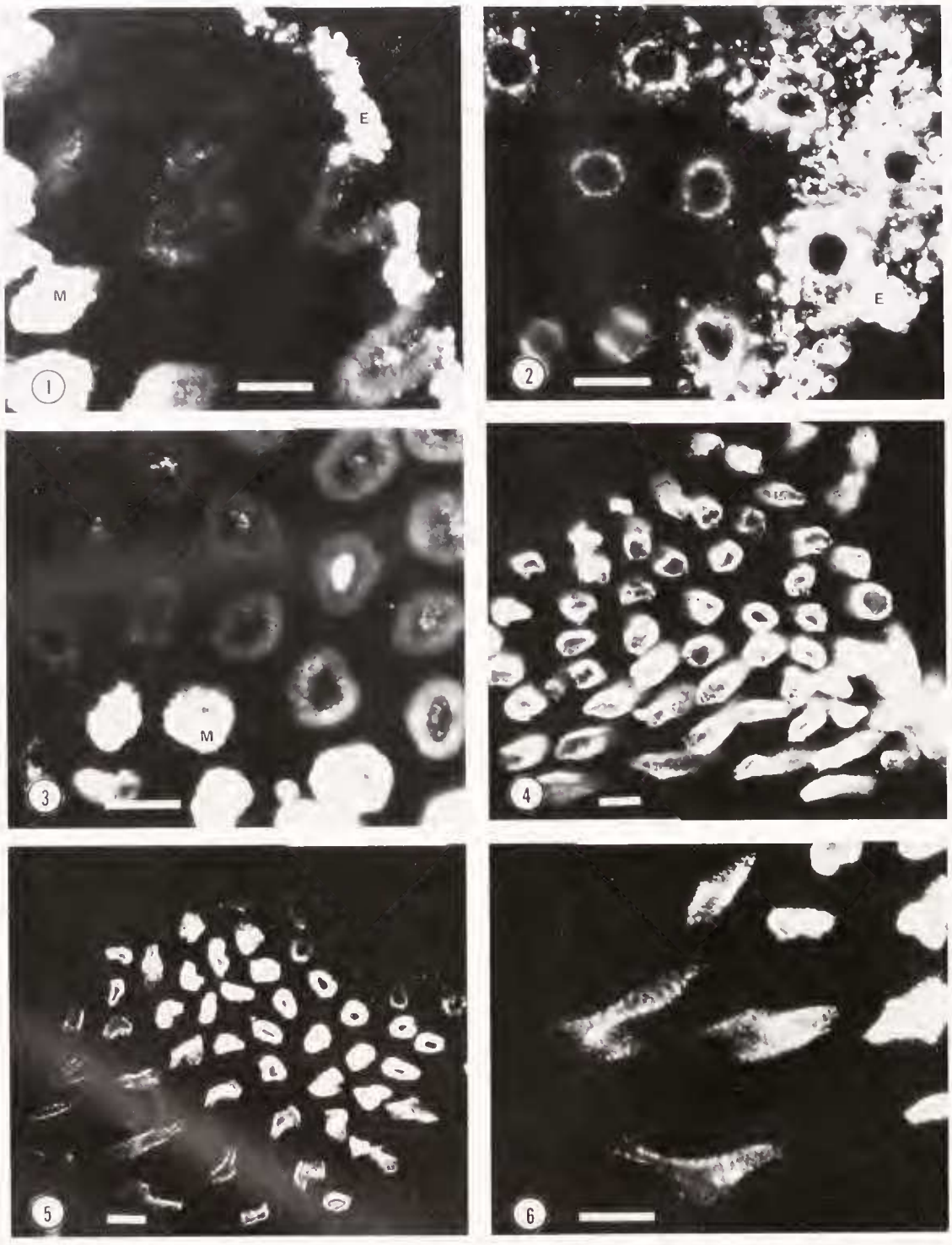

Figure 1. This and the other photographs (Figs. 2–12) show epifluorescent views of unstained sections from two specimens of *Strongylocentrotus droebachiensis* exposed for 5 days to 0.2-μm fluorescent beads suspended in the medium. Unless otherwise stated, the thickness of sectioning is about 20 μm. The photograph shows the opening of madreporite pores to the exterior (middle and lower right). The epidermal cells (E) are filled with beads up to the entrance into the pore canals. Some pore canal cells contain beads, and such cells suddenly become much more abundant midway down the canal (M). All scale bars = 50 μm.

Figure 2. The periphery of the madreporite with the epidermis (E) on right and sections through slightly deeper parts of pores on left. Beads are abundant in pore cells at the entrance of the canals and then become more sparse just inside.

variations in their wet body weights, and be due mainly to the level of inflation of the tube feet, or to adjustments in the position of the peristome. Matched test and control groups of *S. droebachiensis* were selected and observed for several weeks. In one case the animals were fed, and in the other they were not. By using a standardized weighing procedure in which animals were allowed to pre-drain for 2 min on paper towels, daily weight measurements accurate to about 0.1 g were obtained. From a series of such measurements on an individual, a least squares linear regression yielded an average daily change, which was expressed as the percentage of net daily variation from the mean body weight over the period. This deviation was referred to as the "change in body weight index" or ΔWI.

At the conclusion of the experiments, a more specific measurement of the water content of the body parts was made as follows: First, a specimen was inverted for 15 min over a beaker, and any fluid released from its anus was collected. An incision into the body cavity was then made through the equator of the body and the PCF drained out and collected. The animal was then dissected onto tared weighing trays, with the body wall and lantern, the gonads, and the remaining gut separated out. Each collection of tissues was weighed and then dried to constancy (about 24 h) at 75°C, and reweighed. Body water index (BWI) was calculated as the percentage of the wet body weight of the intact animal represented by the drained PCF plus the difference between the total wet and dry weights of parts other than the gut. Gut water index (GWI) similarly was taken as the percentage of the intact animal's wet weight represented by the weight of the collected anal water plus the gut water (difference between wet and dry weights of gut tissue); the water content of any food or feces within the gut was included. The osmotic concentrations of the collected PCF of the animals were also monitored.

Osmotic difference between body fluids and ambient seawater

Finally, osmotic comparisons were made between the body fluids of separate groups of sea urchins and their ambient seawater. For the PCF, about 0.5 ml of fluid was removed from the body cavity with a 26-gauge syringe needle inserted diagonally through the anus, and was immediately placed in a capped microcentrifuge tube. After flushing the syringe with seawater, an equivalent "reference" sample of ambient seawater was taken and placed in a similar tube. Both samples were then centrifuged (5 min) and 10-μl aliquots were then analyzed in a vapor pressure osmometer (Wescor 5500). To compensate for any drift in the instrument, the reference seawater sample was analyzed first with three successive replicates on the sample, which remained sealed in the instrument chamber. The PCF was analyzed next in the same way, followed by another set of analyses on the reference seawater. The mean of the six reference seawater measurements was then subtracted from the mean of the three PCF measurements to yield the osmotic difference. Specimens of *S. franciscanus* were sufficiently large that individual tube feet could be seized with a hemostat, cut off, and drained into a centrifuge tube, and their ambulacral fluid (AF) analyzed in the same way. Since these procedures were designed to measure small osmotic differences between body fluids and ambient seawater (a few mosmol kg^{-1}), the precision of the method was determined by analyzing in the same manner 12 replicates of separate seawater samples substituted for the body fluids. These had a standard deviation of ±2.24 mosmol kg^{-1}.

Statistics

The effects of obstructing the madreporite on ΔWI, BWI, and GWI of test and control animals were evaluated with the Mann-Whitney U test, using the z statistic to estimate significance. A correction for multiple tests for the three procedures may be made using the Bonferroni procedure, in which the selected confidence limit for a single test is divided by the number of tests. In that case, the 95% confidence limit would be set as $P < 0.017$. In the osmotic studies, the mean differences between body fluids and ambient seawater were evaluated with a Student's t test.

Figure 3. Transition to the middle region of the pore canals. In the outer portion, beads are primarily in the lumen (upper right of photograph) or apical part of pore cells (middle and upper left). In the middle region of the pore canal (M) beads are taken up very heavily into all portions of the pore cells.

Figure 4. Middle region of pore canals printed with reduced exposure to show, in detail, the heavy incorporation of beads into the pore canal cells at this level. Section is about 50 μm thick.

Figure 5. Lower portion of madreporite pore canals. As the canals extend (lower left) towards their confluence to form the sub-madreporite ampulla, their cells contain fewer beads than do those in the mid-region of the canal (center).

Figure 6. Cells in the lower region of the pore canals contain fewer beads than those in the middle region (upper right).

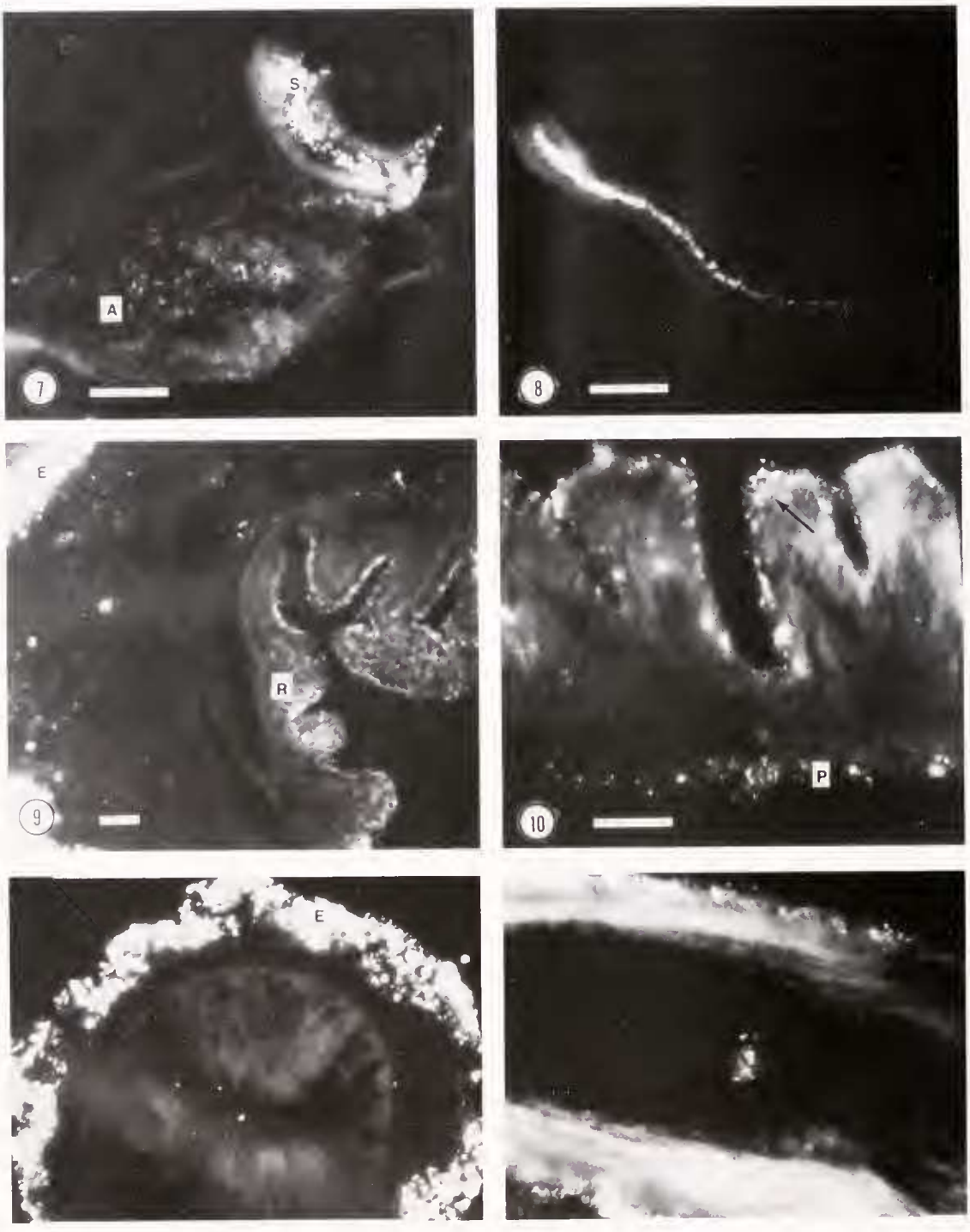

Figure 7. Upper stone canal (s) (near the madreporite) with many beads in its lumen. Some beads may also be seen in the adjacent upper region of the axial organ (A), probably mostly in coelomocytes.

Figure 8. A stringlike congregate of beads and perhaps other material within the lumen of the stone canal in a region below that shown in Fig. 7.

Figure 9. Edge of the rectum (R) with a piece of epidermis (E) showing on upper and lower left. Some beads are on the surface of the rectal lining and a few are in its surrounding peritoneum.

Figure 10. Magnified view of the rectum showing beads on its inner surface (arrow) and some in the peritoneum (P). Beads were not found very often elsewhere in the perivisceral cavity, so these may be in the process of being excreted, perhaps by coelomocytes.

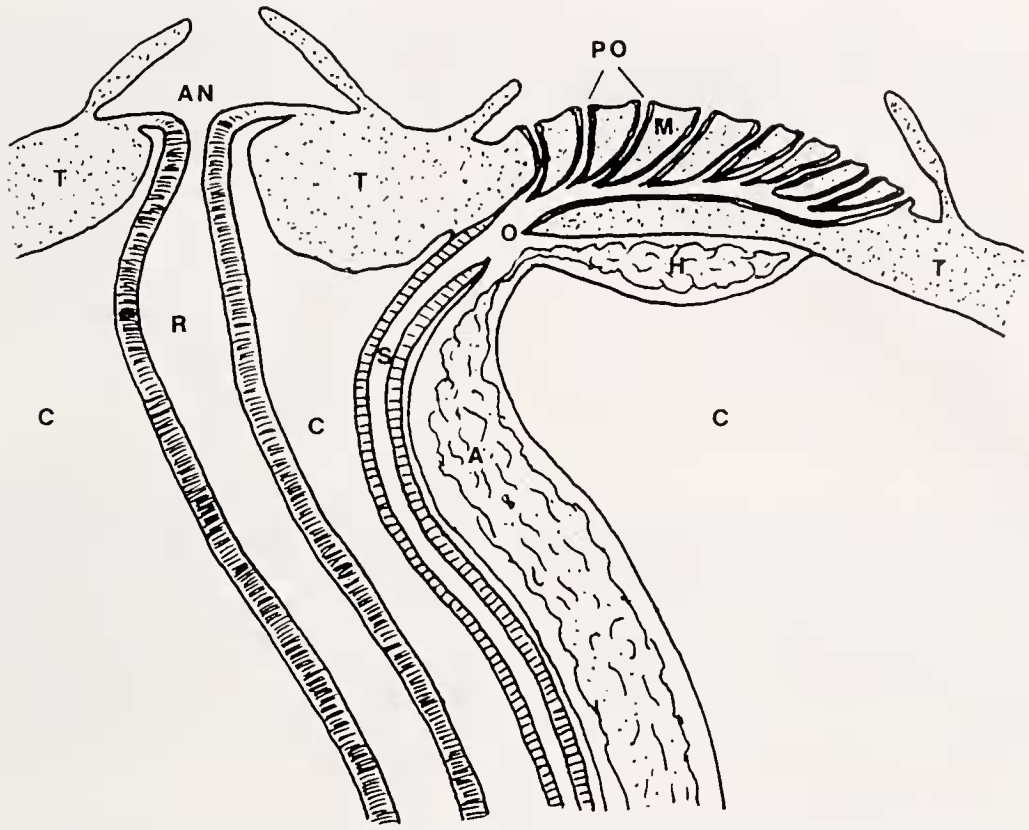

Figure 13. Diagram of the madreporite region. A, axial organ; C, perivisceral coelom; H, head process of axial organ; M, madreporite; O, opening near the upper end of stone canal, between the region of confluence of the pore canals (sub-madreporite ampulla) and the axial sinus; R, rectum; T, test; AN, anus; PO, madreporite pore canal openings.

Results

Uptake of fluorescent microbeads

As was observed in previous studies on asteroids and ophiuroids (Ferguson, 1990b, 1995), microbeads were taken up extensively into the exposed epidermis of the two urchins examined. Substantial numbers were also found within the water vascular system, especially in the madreporite and stone canal (Figs. 1–12). Figure 13 provides a diagrammatic orientation to the madreporite region of the body. In the madreporite, some beads were seen in the lumen of the outer passages of the fine pore canals, and some within the pore cells, most concentrated in their apical portion (Figs. 1–3). In the mid-region of the canals, numerous beads were found throughout the pore cells (Figs. 3–5). At the lower end of the pore canals, the abundance of beads retained diminished markedly as the canals extended towards the region of their confluence (*i.e.*, the sub-madreporite ampulla) and the stone canal beyond (Figs. 5, 6). In the stone canal itself, a thick stringlike aggregate of beads and perhaps other material extended down the lumen (Figs. 7, 8). A few beads were also seen in the upper axial organ (Fig. 7), in the lumen of the rectum (found attached to the madreporite pieces that were sectioned), and in the peritoneum (or coelomocytes) near the rectum (Figs. 9, 10). Some beads were found in the lumen of tube feet, especially in the lower (oral) region of the body (Figs. 11, 12), but they were not plentiful. Many, perhaps most, tube foot sections failed to reveal any beads in the lumen.

Figure 11. Cross section of a tube foot from lower (oral) portion of the body. An abundance of beads is evident in the epidermis (E), but several free beads can also be seen in the central lumen.

Figure 12. Longitudinal section through another tube foot in the same region. Beads are seen in a clump of coelomocytes within the lumen.

Table I

Levels of FID (percentage of medium concentration) in the PCF of madreporite obstructed (Test) and unaltered (Control) Strongylocentrotus pallidus *after incubation in FID seawater*

		Days in medium				
	Weight (g)	1	2	3	4	5
Test	87.5	0	0	0	0	0
	112.4	0				
	155.3	0				
Control	80.7	0	0	0	0	0
	94.7	0				
	116.1	0				

Table III

Effects on indexes of body weight (ΔWI), body water (BWI), gut water (GWI), and PCF osmotic elevation (ΔPCFosm) of groups of unfed Strongylocentrotus droebachiensis *over 21 days: madreporite obstructed (Tests) and unaltered (Controls) specimens (6 pairs)*

	Tests	Controls
Mean wt. (g ± SD)	171.5 ± 27.4	162.0 ± 40.5
ΔWI (% day^{-1} ± SE)	−0.086 ± 0.011*	−0.021 ± 0.015
BWI (% ± SE)	77.00 ± 1.60	79.57 ± 0.98
GWI (% ± SE)	7.98 ± 0.53	6.63 ± 0.40
ΔPCFosm (mosmol kg^{-1} ± SE)	+3.95 ± 1.23	+2.67 ± 0.85

* Difference significant ($P < 0.013$).

Uptake of FID

In three attempts with test and control pairs of *S. pallidus* exposed to high levels of FID in the medium for 24 h (Table I), measurable buildup of the tracer could not be detected in the PCF. In one case, exposure was continued for an additional 4 days, but the substance still could not be observed in the body fluid. I therefore decided that further tests with the FID method were unwarranted.

Long-term effects of madreporite obstruction

A group of madreporite-obstructed specimens of *S. droebachiensis*, and a control group, were observed for 27 days while they were maintained in an aquarium with running seawater and kelp for food. No differences were noticed in the behavior of the two groups. Their tube feet remained active, and animals in both groups were observed to roam the aquarium and climb the sides, feed on the kelp, and rapidly right themselves if upset. No significant differences were measured in the variation of their daily weights (ΔWI) or, at the end of the experiment, in their body water content (BWI) or its osmotic level (Table II). However, the gut water index (GWI) of the "test" animals was found to be significantly ($P < 0.006$) lower than that of the control group (Table II). When a similar experiment was carried out for 21 days on unfed animals, the gut water indexes remained low in both tests and controls, and not significantly different, but the test animals lost weight significantly more rapidly than the controls ($P < 0.013$) (Table III). Otherwise, no differences were noticed in the behavior or well-being of the two groups.

Osmotic differences from ambient seawater

Precise osmotic measurements were made on the PCF of groups of three species (*S. pallidus, S. droebachiensis,* and *S. franciscanus*) and compared to ambient seawater (Table IV). All showed mean osmotic levels significantly above that of their ambient seawater ($P < 0.01$), with a combined mean (±SE) elevation of 2.66 ± 0.39 mosmol kg^{-1}. Clean samples of ambulacral fluid were obtained from the large tube feet of *S. franciscanus*, and they had a mean (±SE) osmotic level (7.94 ± 1.04 mosmol kg^{-1}), significantly above that of the PCF for this species ($P < 0.01$).

Table II

Effects on indexes of body weight (ΔWI), body water (BWI), gut water (GWI), and PCF osmotic elevation (ΔPCFosm) of groups of Strongylocentrotus droebachiensis *fed* ad libitum *for 27 days: madreporite obstructed (Tests) and unaltered (Controls) specimens (11 pairs)*

	Tests	Controls
Mean wt. (g ± SD)	172.6 ± 24.4	178.0 ± 31.2
ΔWI (% day^{-1} ± SE)	+0.010 ± 0.011	−0.014 ± 0.011
BWI (% ± SE)	77.99 ± 0.75	77.36 ± 0.56
GWI (% ± SE)	13.21 ± 0.55*	16.27 ± 0.86
ΔPCFosm (mosmol kg^{-1} ± SE)	+2.16 ± 0.84	+2.77 ± 0.68

* Difference significant ($P < 0.006$).

Table IV

Osmotic difference between body fluids of sea urchins and their ambient seawater

Species	Fluid	n	mosmol kg^{-1} ± SE
S. pallidus	PCF	12	+2.86 ± 0.69
S. droebachiensis	PCF	12	+2.68 ± 0.77
S. franciscanus	PCF	12	+2.44 ± 0.59
S. franciscanus	AF	12	+7.94 ± 1.04
Seawater*		12	−0.54 ± 0.65

* Repetitive samples analyzed just like body fluids (all body fluids are significantly different from seawater ($P < 0.01$)).

Discussion

These experiments with fluorescent microbeads and soluble tracer (FID) show that in contrast to previous work in asteroids (Ferguson 1989, 1990b) entry of seawater into the madreporite of echinoids is very limited. The continued, apparently unimpaired activity of tube feet even after 27 days of madreporite occlusion further indicates that these structures are inflated mainly by some other means. Certainly their elevated osmotic AF (+7.94 mosmol kg^{-1} in *S. franciscanus*) must make a contribution, although redistribution of fluids between body compartments may also be involved. Likewise, the failure to detect significant amounts of FID in the PCF shows that there can be but little transmadreporitic inflow of seawater to generate that fluid. If minor entry did occur, it was completely masked by the natural cleansing processes that must exist within the animals and the limits of sensitivity of the method. In a parallel study in which this method was used on the starfish *Echinaster graminicola*, uptake of FID into the PCF was easily measured (in spite of evident fluid cleansing) and found to be about 1.5 $\mu l\ g^{-1}\ h^{-1}$, out of a total madreporite influx of 2.3 $\mu l\ g^{-1}\ h^{-1}$ (Ferguson, 1989). The transmadreporite influx of seawater in starfish thus appears to be directed mainly at keeping the flexible body inflated, a process that is largely unnecessary in sea urchins. Moreover, sea urchins do not have Tiedemann's bodies, which may be asteroid specializations for passing fluid to the PCF. With their rigid test obviating much of the need for fluid volume control, sea urchins appear to rely mainly on a higher PCF osmotic level (+2.66 mosmol kg^{-1} reported here) than seen in most asteroids (Ferguson, 1990a), and on a net negative hydrostatic pressure reportedly produced within the test by the movement of the lantern and peristome (Ellers and Telford, 1992).

Is there any consistent flow of seawater at all into the sea urchin madreporite? Recent work by Tamori *et al.* (1996) on isolated madreporite pieces indicates that the pores usually maintain an inward flow that is regulated by changes in their diameter. Cilia within the pores tended to eject particles aborally. The present work shows that a small proportion of minute particles (beads) suspended in the medium can still enter the pore canals of intact animals. These particles are extensively trapped, especially by middle pore cells (Figs. 1–6). Many of the particles that succeed in passing through the length of the pore canals become entangled in a string of material within the lumen of the stone canal (Figs. 7, 8), which is lined with ciliated cells filled with granules (Rehkämper and Welsch, 1988). Nevertheless, a few particles pass by these obstacles and are transported to the lumen of distal tube feet, where they are finally phagocytized by coelomocytes (Figs. 11, 12). Particles and phagocytic debris also accumulate to some extent in the axial gland, near the rectum, which may be a site of phagocytic egress (Figs. 7, 9, 10). These observations lead to the conclusion that a very slow bulk flow of seawater passes through the madreporite and down the stone canal to the tube feet, and that the pore cells help to remove foreign material from this entering stream.

Although the FID study was not sensitive enough to show such a slow flow, other data support its existence. In working with the starfish *Pisaster ochraceus*, the transmadreporite seawater influx was estimated from the rate of weight loss in animals with an obstructed madreporite (Ferguson, 1992). That value was 2.2 $\mu l\ g^{-1}\ h^{-1}$ (which was close to the rate determined from the FID studies on *Echinaster* (Ferguson, 1989)). Using a similar approach, madreporite seawater influx in *S. droebachiensis* can be roughly estimated by comparing the mean weight loss differences (ΔWI) in test and control animals (Table III). If all the difference between the two groups was due to inhibited madreporite influx, that influx would amount to about 0.3 $\mu l\ g^{-1}\ h^{-1}$; it is equal to a maximum total inflow of not more than 0.13 ml of seawater a day into the madreporite of a 200-g sea urchin. This rate is at least 70 times lower than the ones calculated for starfish. Although this approach is not very precise, the order of magnitude of the figure obtained seems consistent with differences seen in the microbead observations, negative FID experiments, and other observations, including the negative findings of Fechter (1965).

Does such a low rate of madreporite seawater entry have any functional significance? The long-term weight-change studies (Tables II and III) give some indication that it might, eventually. Although the differences resulting from madreporite obstruction were small and not noticeable in the behavior of the animals, unfed test specimens gradually became unable to maintain body weight, whereas fed test specimens had a slightly higher gut water index. The reasons for these effects are unknown, but possibilities include a gradually increasing deficit of AF that may have limited the number of tube feet that could be used at any one time; or a reduced PCF volume that might have hampered the animal's ability to protrude its lantern apparatus. These kinds of effects could be related. The water vascular system is connected indirectly to the perivisceral coelom through an opening between the upper end of the stone canal and the adjacent axial sinus (Fig. 13) (*cf.*, Jangoux and Schaltin, 1977; Bachmann and Goldschmid, 1978). So deficiencies in fluid content in one compartment might affect the functions of the other, given enough time. Thus, this limited fluid uptake appears to "top off" a fluid balance that is largely met by the osmotic differences between the body fluids and the surrounding seawater, and by net negative periodic hydrostatic pressures in the perivisceral coelom.

The madreporite may have other functions that are unrelated to fluid volume regulation. These include chemosensing, a passage for entry of pheromonal stimulants, and excretory roles. None of these has as yet been convincingly demonstrated.

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